Time course of change in vasodilator function and capacity in response to exercise training in humans

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Studies of the impact of exercise training on arterial adaptation in healthy subjects have produced disparate results. It is possible that some studies failed to detect changes because functional and structural adaptations follow a different time course and may therefore not be detected at discrete time points. To gain insight into the time course of training-induced changes in artery function and structure, we examined conduit artery flow mediated dilatation (FMD), an index of nitric oxide (NO)-mediated artery function, and conduit dilator capacity (DC), a surrogate marker for arterial remodelling, in the brachial and popliteal arteries of 13 healthy male subjects (21.6 ± 0.6 years) and seven non-active controls (22.8 ± 0.2 years) studied at 2-week intervals across an 8-week cycle and treadmill exercise training programme. Brachial and popliteal artery FMD and DC did not change in control subjects at any time point. FMD increased from baseline (5.9 ± 0.5%) at weeks 2 and 4 (9.1 ± 0.6, 8.5 ± 0.6%, respectively, $P < 0.01$), but returned towards baseline levels again by week 8 (6.9 ± 0.7%). In contrast, brachial artery DC progressively increased from baseline (8.1 ± 0.4%) at weeks 2, 4, 6 and 8 (9.2 ± 0.6, 9.9 ± 0.6, 10.0 ± 0.5, 10.5 ± 0.8%, $P < 0.05$). Similarly, popliteal artery FMD increased from baseline (6.2 ± 0.7%) at weeks 2, 4 and 6 (9.1 ± 0.6, 9.5 ± 0.6, 7.8 ± 0.5%, respectively, $P < 0.05$), but decreased again by week 8 (6.5 ± 0.6%), whereas popliteal DC progressively increased from baseline (8.9 ± 0.4%) at week 4 and 8 (10.5 ± 0.7, 12.2 ± 0.6%, respectively, $P < 0.05$). These data suggest that functional changes in conduit arteries occur rapidly and precede arterial remodelling *in vivo*. These data suggest that complimentary adaptations occur in arterial function and structure and future studies should adopt multiple time point assessments to comprehensively assess arterial adaptations to interventions such as exercise training in humans.

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Exercise training typically improves arterial function and structure in subjects with prevailing cardiovascular risk factors or disease (Green *et al.* 2004). However, in healthy subjects, the impact of large muscle group dynamic exercise training on arterial function remains equivocal. For example, 4 weeks of cycling in healthy men improved nitric oxide (NO)-mediated forearm resistance vessel function (Kingwell *et al.* 1997*b*), whereas, in healthy middle-aged men, 8 weeks of circuit training did not affect vascular function (Maiorana *et al.* 2001). Brachial artery flow mediated dilatation (FMD) was enhanced following a 10-week programme of daily aerobic and anaerobic exercise training in young military recruits (Clarkson *et al.* 1999), whereas other studies have observed no such change in otherwise healthy subjects (Ostergard *et al.* 2006; Thijssen *et al.* 2007).

One possible explanation for these apparently disparate findings in healthy subjects relates to the time course of change in artery function and structure in humans. The hypothetical suggestion is that, whilst short-term exercise training enhances eNOS and NO bioactivity (Delp *et al.* 1993; Sessa *et al.* 1994; Sun *et al.* 1994; Delp & Laughlin, 1997), extended training induces structural changes which result in an increase in arterial size (Brown, 2003; Prior *et al.* 2003). This paradigm suggests that 'structural' adaptation or arterial remodelling normalizes shear rate levels and may result in NO-mediated endothelial function returning towards initial levels. Whilst some indirect evidence supports this hypothesis in humans (Green *et al.* 2004), it has not previously been directly examined.

Nonetheless, as originally pointed out by Laughlin and colleagues (Laughlin, 1995; Laughlin *et al.* 2003), animal

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Table 1. Baseline characteristics of exercise training subjects ($n = 13$) and controls ($n = 7$) before (0 weeks) and after the exercise intervention (8-weeks)

	Exercise training ($n = 13$)		Controls ($n = 7$)	
	0 weeks	8 weeks	0 weeks	8 weeks
Age (years)	22 ± 2		23 ± 4	
Weight (kg)	82 ± 12		78 ± 6	
Height (cm)	181 ± 6		178 ± 6	
Total body fat (%)	18.6 ± 6.7	18.0 ± 6.7	18.2 ± 4.2	18.2 ± 4.4
Total lean mass (kg)	64 ± 6	65 ± 7	60 ± 6	60 ± 6
$\dot{V}_{O_2,max}$ (ml kg^{-1} min^{-1})	46.2 ± 6.2	50.3 ± 5.2*	49.6 ± 7.5	49.1 ± 8.2
SBP (mmHg)	113 ± 9.9	112 ± 8.9	115 ± 7.1	115 ± 7.2
DBP (mmHg)	63 ± 8.4	63 ± 8.0	72 ± 7.2	72 ± 7.5
MAP (mmHg)	79 ± 8.3	79 ± 7.0	86 ± 6.9	86 ± 7.2
HR (bpm)	69 ± 1.2	68 ± 8.0	68 ± 2.0	69 ± 1.7

*Significant from pretraining at $P < 0.05$ (paired t test) Values are means and ± S.D.; $\dot{V}o_{2,max}$, maximal oxygen consumption; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate.

studies provide some support for the above contention. Short-term exercise training (e.g. 7–10 days) enhanced endothelium-dependent dilator responses in dog and rat arterioles (Wang *et al.* 1993; Koller *et al.* 1995) and also in pig conduit vessels (McAllister & Laughlin, 1997). In addition, 10 days of training increased nitrite and NO production and eNOS gene expression in dog coronary arterioles (Sessa *et al.* 1994), while 2–4 weeks of training improved vascular function and increased eNOS protein levels in rat skeletal muscle arterioles (Sun *et al.* 1994) and aorta (Delp *et al.* 1993; Delp & Laughlin, 1997). In contrast, animal studies that used longer durations of exercise training (e.g. 16–20 weeks), have demonstrated no change in NO-dependent vasodilation (McAllister *et al.* 1996; Kingwell *et al.* 1997*a*) or eNOS expression (Johnson & Laughlin, 2000). Taken together, these data suggest that improvement in endothelium-dependent vasodilator responses and eNOS expression in the periphery may be a transient phenomenon that is lost with longer-term training. Interestingly, prolonged exercise training in various animal models enlarges the diameter of arteries (Leon & Bloor, 1968; Wyatt & Mitchell, 1978; Kramsch *et al.* 1981; Lash & Bohlen, 1992). Indeed, it is well established that exercise training induces structural enlargement of conduit vessels (Rodbard, 1975; Zamir, 1977; Guyton & Hartley, 1985; Langille *et al.* 1989; Tronc *et al.* 1996; Lloyd *et al.* 2001; Prior *et al.* 2003), which is reported to be endothelium and NO dependent (Kamiya & Togawa, 1980; Langille & O'Donnell, 1986; Zarins *et al.* 1987; Gibbons & Dzau, 1994; Rudic *et al.* 1998; Prior *et al.* 2003). Such structural changes may act to supplant the acute vasodilator functional responses described above, reinforcing the proposal that functional change may be superseded by structural shear stress normalization (Laughlin, 1995; Laughlin *et al.* 2003; Green *et al.* 2004).

Despite this evidence from animal studies, no studies have specifically addressed the issue of the time course of change in conduit artery structure and function in humans in response to exercise training. In the present study, we measured conduit artery flow mediated dilatation (FMD), an assay of endothelium-dependent, largely NO-mediated vasodilatation (Joannides *et al.* 1995; Kooijman *et al.* 2008), every 2 weeks across an 8-week exercise training programme in young healthy subjects. In addition we measured the conduit artery vasodilator response to an ischaemic exercise stimulus, which elicits near maximal vasodilatation (Naylor *et al.* 2005), as an indirect measure of vasodilator capacity.

Methods

Twenty healthy recreationally active male volunteers were recruited and allocated to 8 weeks of exercise training ($n = 13$) or a similar period in which they continued their normal physical activity levels ($n = 7$, controls) (Table 1). Subjects were young and healthy; none reported having been diagnosed with cardiovascular disease, diabetes, insulin resistance or cardiovascular risk factors such as hypercholesterolaemia or hypertension. Subjects who smoked or were on medications of any type were excluded. The study procedures were approved by the Ethics Committee of Liverpool John Moores University and adhered to the *Declaration of Helsinki*. Written informed consent was gained from all participants prior to the experimental procedures.

Experimental design

Before the intervention, subjects reported 3 times to the laboratory for assessment of brachial and popliteal artery

function and vasodilator capacity and for examination of body composition and physical fitness level. Subsequently, subjects were randomized to either 8 weeks of exercise training or a control period. Assessment of brachial and popliteal artery function and vasodilator capacity was repeated at 2-week intervals across an 8-week intervention in all subjects. Finally, brachial and popliteal artery function and vasodilator capacity assessments, as well as body composition and aerobic capacity were determined at the end of the 8-week intervention.

Visits 1 and 2: assessment of vascular function and vasodilator capacity

Vascular function assessments were conducted over two testing days in a quiet, temperature controlled environment. As repeated measurements were performed, each test for a given subject was performed at the same time of day. Subjects were asked prior to the first testing session to fast for 6 h, abstain from alcohol and caffeine for 12 h, and not to perform any exercise for 24 h.

Brachial artery vascular function. Subjects rested in the supine position for a period of at least 15 min to facilitate baseline assessment of heart rate and blood flow. Heart rate and mean arterial pressure were determined from an automated sphygmomanometer (Dinamap; GE Pro 300V2, Tampa, FL, USA). To examine brachial artery FMD, the arm was extended and positioned at an angle of ∼80 deg from the torso. A rapid inflation and deflation pneumatic cuff (D. E. Hokanson Inc., Bellevue, WA, USA) was positioned on the forearm of the imaged arm immediately distal to the olecranon process to provide a stimulus to forearm ischaemia (Corretti *et al.* 2002). A 10 MHz multifrequency linear array probe attached to a high resolution ultrasound machine (Aspen, Acuson, Mountain View, CA, USA) was used to image the brachial artery in the distal one-third of the upper arm. When an optimal image was obtained, the probe was held stable and the ultrasound parameters were set to optimize the longitudinal, B-mode images of lumen–arterial wall interface. Continuous Doppler velocity assessment was also obtained using the Aspen, and was collected using the lowest possible insonation angle (always < 60 deg), which did not vary during each study. Baseline images were recorded on an S-VHS video cassette recorder (SVO-9500 MDP, Sony, Tokyo, Japan) for 1 min. The forearm cuff was inflated (> 200 mmHg) for 5 min. Diameter and flow recordings resumed 30 s prior to cuff deflation and continued for 3 min thereafter.

Brachial artery vasodilator capacity. After measuring brachial artery vascular function, a 15 min resting period was observed before a 1 min baseline recording of diameter, flow and shear rate. The occluding cuff was positioned above the imaged part of the conduit artery, i.e. proximally on the upper arm, which was inflated to > 200 mmHg for 5 min. During the middle 3 min, ischaemic handgrip exercise was performed. Diameter and flow recordings resumed 30 s prior to cuff deflation and continued for 3 min thereafter. This protocol results in a near maximal dilatation of the brachial artery in humans (Naylor *et al.* 2005) and provides a valid index of peak vasodilator capacity.

Popliteal artery vascular function and vasodilator capacity. Subsequently, subjects were placed in the prone position with the knee at an angle of approximately 20 deg for a period of 15 min to measure popliteal artery function and vasodilator capacity. First, a pneumatic cuff (D. E. Hokanson Inc.) was positioned on the imaged leg immediately distal to the popliteal fossa to provide the 5 min ischaemic stimulus. The popliteal artery was imaged above the occluding cuff just behind the knee. Measurement of popliteal artery vascular function was performed as described above for the brachial artery. After another resting period of 15 min, popliteal artery vasodilator capacity was examined. The occluding cuff was placed above the imaged artery at the distal one-third of the thigh. During the 5 min ischaemia, 3 min of isotonic plantar flexion exercise was performed. Measurement of popliteal artery diameter and velocity were performed as described above.

Day 3: baseline assessments

Body composition and physical fitness. A dual-energy X-ray absorptiometry (DEXA) scanner (Hologic QDR Series Discovery A, Bedford, MA, USA) was used before and after the 8-week training period to determine body fat, bone mineral tissue and residual tissue in each subject. Exercise testing was undertaken on a treadmill ergometer (H/P/Cosmos, Pulsar 4.0, Nussdorf-Traunstein, Germany) in a temperature controlled room. The test was performed with initial workload set at 6 km h^{-1} and step-wise increments in speed (speed increased by 2 km h^{-1} every 2 min until 16 km h^{-1}) and slope (2% every minute when 16 km h^{-1} is reached) until volitional exhaustion. The volume of oxygen consumption ($\dot{V}_{O_2}$) during exercise was calculated from minute ventilation, measured using a pneumotach and simultaneous breath-by-breath analysis of expired gas fractions (Medgraphics CPX/D and Ultima CardiO_2 systems, MN, USA). Gas analysers and flow probes were calibrated before each test. Oxygen consumption was recorded during the final 40 s of each stage of the test and expressed relative to body weight (ml kg^{-1} min^{-1}). Peak oxygen consumption was calculated as the highest

consecutive 10 s period of gas exchange data occurring in the last minute before volitional exhaustion, which generally occurred due to leg fatigue or breathlessness.

Exercise training protocol

Exercise training was performed over an 8-week period with subjects visiting the laboratory 3 times a week. Each session was supervised and consisted of 15 min of running and 15 min cycle exercise at 80% heart rate reserve (HRR), which was calculated from each subject's maximum and resting heart rate. A Polar heart rate monitor (Polar Electro Oy, Kempele, Finland) was used to continuously monitor heart rate. During the 8 weeks, exercise workload was adapted to maintain 80% HRR during each training session. Over the 8 weeks of exercise training, there was 90% adherence with the training sessions. Subjects were instructed to minimize incidental handgrip exercise during the predominantly lower limb training. The rationale for this instruction is that we aimed to determine whether changes in vascular function or remodelling were limited to the active vessel bed (lower limbs) or generalized to regions not directly involved in the training stimulus.

Brachial and popliteal artery diameter and blood flow analysis

Post-test analysis of brachial and popliteal artery diameters was performed using custom-designed edge-detection and wall-tracking software, which is largely independent of investigator bias (Woodman *et al.* 2001). Briefly, the video signal was taken directly from the ultrasound machine and, using an IMAQ-PCI-1407 card, was encoded and stored as a digital DICOM file on the PC. Subsequent software analysis of these data was performed at 30 Hz using an icon-based graphical programming language and toolkit (LabVIEW 6.02, National Instruments, Austin, TX, USA). The initial phase of image analysis involved the identification of regions of interest (ROI) on the first frame of every individual study. These ROIs allowed automated calibration for diameters on the B-mode image and velocities on the Doppler strip. An ROI was then drawn around the optimal area of the B-mode image and within this ROI a pixel-density algorithm automatically identified the angle-corrected near and far-wall e-lines for every pixel column within the ROI. The algorithm begins by dividing the ROI into an upper half, containing the near wall lumen–intima interface, and a lower half containing the far wall interfaces. The near-wall intimal edge is identified by a Rake routine that scans from the bottom to the top of the upper half of the ROI. The position of the edge is established by determining the point where the pixel intensity changes most rapidly. Typical B-mode ROIs therefore contained approximately 200–300 diameter measures per frame, the average of which was calculated and stored. This process occurred at 30 frames per second.

A final ROI was drawn around the Doppler waveform and automatically detected the peak of the waveform. The mean diameter measures derived from within the B-mode ROI (above) were then synchronized with the velocity measure derived from the Doppler ROI at 30 Hz. Ultimately, from this synchronized diameter and velocity data, blood flow (the product of lumen cross-sectional area and Doppler velocity (v)) and shear rate (4 times velocity divided by diameter) were calculated at 30 Hz. All data were written to file and retrieved for analysis in a custom designed analysis package. We have shown that reproducibility of diameter measurements using this semi-automated software is significantly better than manual methods, reduces observer error significantly, and possesses an intraobserver CV of 6.7% (Woodman *et al.* 2001). Furthermore, our method of blood flow assessment is closely correlated with actual flow through a 'phantom' arterial flow system (Green *et al.* 2002).

Data analysis

Baseline diameter, flow and shear rate were calculated as the mean of data acquired across the 1 min preceding the cuff inflation period. Peak diameter following cuff deflation was automatically detected according to an algorithm which identified the maximum bracket of data subsequent to performance of a moving window smoothing function. This smoothing routine calculates the median value from 100 consecutive samples, before the window shifts to the next bracket of data, which shares 20% overlap with the preceding bracket. The maximum value of all the calculated median values is then automatically detected and chosen to represent the peak of the postdeflation artery diameter curve. FMD was calculated as the percentage rise of this peak diameter from the preceding baseline diameter. The time to peak diameter (in seconds) was calculated from the point of cuff deflation to the maximum postdeflation diameter. Calculation of FMD and time to peak were therefore observer-independent and based on standardized algorithms applied to data which had undergone automated edge-detection and wall-tracking.

In accordance with recent findings (Pyke & Tschakovsky, 2007), we calculated the shear rate stimulus responsible for endothelium-dependent FMD. The postdeflation shear rate data, derived from simultaneously acquired velocity and diameter measures at 30 Hz, were exported to a spreadsheet and the area under the shear rate curve (AUC) calculated for data up to the point of maximal post-deflation diameter (FMD) for each individual using the trapezoid rule. Peak blood flow (ml min^{-1}) in response to the DC stimuli, calculated from diameter

Table 2. Brachial artery characteristics throughout an 8-weeks exercise intervention ($n = 13$) or control period ($n = 7$), measured at 2-week intervals

	Week 0	Week 2	Week 4	Week 6	Week 8	ANOVA
Exercise training ($n = 13$)						
Diameter (mm)	4.2 ± 0.4	4.1 ± 0.6	4.0 ± 0.6	4.1 ± 0.4	4.3 ± 0.6	0.89
FMD (%)	5.9 ± 1.9	9.1 ± 2.1*	8.4 ± 2.3*	7.6 ± 1.7	6.9 ± 2.4	0.001
FMD (mm)	0.27 ± 0.06	0.31 ± 0.13	0.38 ± 0.18	0.31 ± 0.07	0.29 ± 0.19	0.12
AUC_{SR}	11596 ± 5236	19316 ± 13924	17759 ± 10655	15749 ± 7331	16730 ± 9341	0.42
DC (%)	8.1 ± 1.4	9.2 ± 2.6*	9.9 ± 2.2*	10.4 ± 1.5*	10.5 ± 2.3*	0.02
DC (mm)	0.35 ± 0.12	0.37 ± 0.07	0.40 ± 0.10	0.37 ± 0.07	0.41 ± 0.13	0.40
Resting flow (ml min^{-1-1})	63 ± 28	62 ± 39	63 ± 41	64 ± 27	64 ± 17	0.99
Peak RH flow (DC) (ml min^{-1-1})	497 ± 313	616 ± 232	578 ± 255	648 ± 274	634 ± 248	0.47
Flow AUC (DC) (ml s^{-1})	606 ± 247	656 ± 318	645 ± 315	651 ± 341	651 ± 281	0.95
Controls ($n = 7$)						
Diameter (mm)	4.2 ± 0.7	4.3 ± 0.4	4.2 ± 0.9	4.3 ± 0.3	4.1 ± 0.9	0.71
FMD (%)	6.8 ± 2.1	7.3 ± 3.2	6.9 ± 1.0	6.8 ± 2.4	6.8 ± 1.2	0.99
FMD (mm)	0.27 ± 0.66	0.26 ± 0.10	0.27 ± 0.13	0.28 ± 0.07	0.24 ± 0.10	0.91
AUC_{SR}	17602 ± 11185	16303 ± 11924	15158 ± 20926	15324 ± 5203	15415 ± 12568	0.99
DC (%)	8.7 ± 3.6	9.1 ± 2.9	9.0 ± 3.1	9.2 ± 2.9	9.4 ± 3.0	0.99
DC (mm)	0.32 ± 0.10	0.33 ± 0.10	0.37 ± 0.12	0.37 ± 0.01	0.32 ± 0.01	0.24
Resting flow (ml min^{-1})	65 ± 27	64 ± 26	64 ± 42	64 ± 10	64 ± 17	0.99
Peak RH flow (DC) (ml min^{-1})	506 ± 200	508 ± 170	506 ± 244	508 ± 186	508 ± 150	1.00
Flow AUC (DC) (ml s^{-1})	616 ± 255	613 ± 258	618 ± 419	612 ± 150	612 ± 162	1.00

*Significant from baseline at $P < 0.05$. Values are means and ± S.D.; S.D., standard deviation; n, no of subjects; FMD, flow-mediated dilatation; AUC_{SR}, shear rate area under the curve; DC, dilator capacity; RH, reactive hyperemia

Table 3. Popliteal artery characteristics throughout an 8-weeks exercise intervention ($n = 13$) or control period ($n = 7$), measured at 2-week intervals

	Week 0	Week 2	Week 4	Week 6	Week 8	ANOVA
Exercise training ($n = 13$)						
Diameter (mm)	4.9 ± 0.1	4.9 ± 0.1	4.9 ± 0.8	5.1 ± 0.5	5.0 ± 0.1	0.98
FMD (%)	6.2 ± 2.6	9.2 ± 2.0*	9.5 ± 2.2*	7.8 ± 1.7*	6.6 ± 2.2	0.00
FMD (mm)	0.31 ± 0.18	0.45 ± 0.13	0.44 ± 0.15	0.42 ± 0.14	0.31 ± 0.10	0.40
AUC_{SR}	9307 ± 6102	11750 ± 9748	6526 ± 4029	6354 ± 3288	7295 ± 5883	0.29
DC (%)	8.9 ± 1.4	9.3 ± 3.1	10.5 ± 2.4*	10.5 ± 3.4*	12.2 ± 2.8*	0.02
DC (mm)	0.45 ± 0.11	0.44 ± 0.20	0.43 ± 0.13	0.49 ± 0.20	0.49 ± 0.18	0.76
Resting flow (ml min^{-1-1})	52 ± 35	53 ± 29	53 ± 19	53 ± 35	53 ± 24	0.99
Peak RH flow (DC) (ml min^{-1})	484 ± 355	491 ± 255	515 ± 118	596 ± 262	546 ± 331	0.72
Flow AUC (DC) (ml s^{-1})	552 ± 216	584 ± 302	626 ± 189	611 ± 387	489 ± 264	0.71
Controls ($n = 7$)						
Diameter (mm)	5.2 ± 0.8	4.8 ± 0.8	4.5 ± 0.1	4.9 ± 0.8	4.7 ± 0.1	0.73
FMD (%)	7.5 ± 3.8	7.1 ± 3.0	7.9 ± 3.7	7.8 ± 2.1	7.8 ± 3.4	0.99
FMD (mm)	0.36 ± 0.23	0.32 ± 0.13	0.37 ± 0.22	0.36 ± 0.19	0.33 ± 0.15	0.99
AUC_{SR}	11773 ± 10554	9839 ± 6797	8537 ± 7007	10122 ± 5644	9102 ± 5921	0.93
DC (%)	6.2 ± 1.9	6.2 ± 2.1	6.8 ± 2.2	6.3 ± 2.8	6.5 ± 2.0	0.81
DC (mm)	0.26 ± 0.12	0.23 ± 0.07	0.27 ± 0.11	0.24 ± 0.15	0.26 ± 0.10	0.90
Resting flow (ml min^{-1})	48 ± 26	49 ± 52	48 ± 38	49 ± 17	49 ± 26	0.99
Peak RH flow (DC) (ml min^{-1})	414 ± 222	415 ± 160	414 ± 271	413 ± 179	416 ± 137	1.00
Flow AUC (DC) (ml s^{-1})	426 ± 257	425 ± 107	425 ± 170	423 ± 215	423 ± 104	1.00

*Significant from baseline at $P < 0.05$. Values are means and ± S.D.; S.D., standard deviation; n, no of subjects; FMD, flow-mediated dilatation; AUC_{SR}, shear rate area under the curve; DC, dilator capacity; RH, reactive hyperaemia.

(cross-sectional area) and velocity data, was recorded as the highest mean blood flow across a 10 second period following cuff deflation, this epoch being selected on the basis of previous measures collected using plethysmography. We also calculated cumulative flow AUC (ml s^{-1}), from the point of cuff deflation to the point of occurrence of peak artery diameter, in each subject (Tables 2 and 3).

Statistics

Statistical analyses were performed using SPSS 14.0 (SPSS Inc., Chicago, IL, USA) software. All data are reported as the mean (S.D.) unless stated otherwise, while statistical significance was assumed at $P < 0.05$. Repeated measures ANOVA (with time and group as independent factors) and *post hoc* analysis (*t* tests and least significant differences tests for multiple comparisons) were used to assess the changes in brachial and popliteal artery function and structure throughout the 8-week intervention period in the trained as well as in the control group.

Results

Subject characteristics were not different between groups (Table 1). Maximal oxygen consumption was significantly increased after 8 weeks in the exercise training group, but not in the control group (Table 1). Other subject characteristics did not change in either group (Table 1).

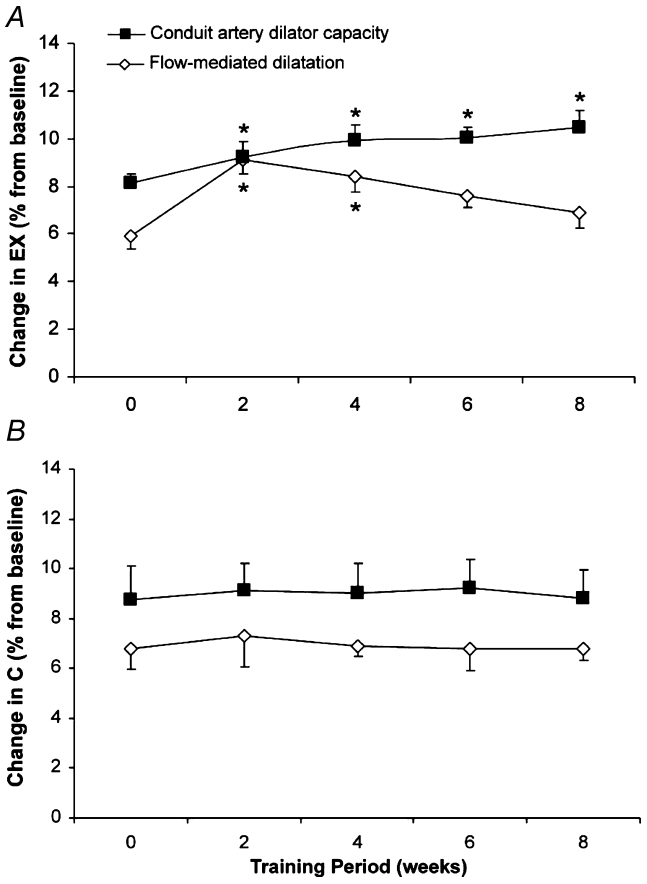

Figure 1. Relative change in brachial artery diameter from baseline (as a percentage) in response to 5 min ischaemia (flow-mediated dilatation, FMD, ◇) or 5 min ischaemic exercise (vasodilator capacity, DC, ■)
Data are presented before, after and at 2-week intervals throughout an 8-week intervention in exercise trained subjects ($n = 13$, EX) (*A*), and controls ($n = 7$, C) (*B*). Error bars represent S.E.M. $^*P < 0.05$.

Brachial artery function and vasodilator capacity

Function. Baseline brachial artery diameter in the exercise-trained subjects did not change during training (Table 2). Exercise training induced a change in brachial artery FMD (ANOVA, $P < 0.05$) (Fig. 1*A*). Brachial artery FMD at 2 and 4 weeks was significantly higher compared with baseline, but returned to near baseline values at week 8 (Fig. 1*A*). The area under the curve for shear rate from cuff deflation to the point of peak diameter (AUC_{SR}) did not change with training (Table 2).

Vasodilator capacity. Brachial artery DC, presented as relative change from baseline, showed a progressive increase across the training intervention period (Fig. 1*A*) ($P < 0.05$).

Popliteal artery function and vasodilator capacity

Function. Baseline popliteal artery diameter in the exercise intervention group did not change over the 8-week intervention (Table 3). Training increased popliteal artery FMD, with significant increases from baseline at weeks 2, 4 and 6 (Fig. 2*A*). The AUC_{SR} from cuff deflation to the point of peak diameter (AUC_{SR}) did not change during training (Table 3).

Vasodilator capacity. As was the case for brachial artery, popliteal artery DC, presented as relative change from baseline, demonstrated a progressive increase across the training intervention period (Fig. 2*A*) ($P < 0.05$).

Control subjects did not differ from the exercise training group at baseline (Tables 2 and 3). Neither brachial nor popliteal artery FMD and DC responses differed across the 8-week intervention period in the inactive control group (Figs 1*B* and 2*B*, respectively).

Discussion

This is the first study in humans to examine the time course of vascular adaptations in conduit artery function and vasodilator capacity across an exercise training programme *in vivo*. We found that brachial artery endothelial function increased after short-term exercise training, but returned to near normal levels after a longer training period. Brachial artery conduit artery dilator capacity, a surrogate measure for arterial remodelling (Naylor *et al.* 2005), gradually increased across the 8-week training period. These findings suggest that exercise-induced functional changes in conduit arteries precede structural adaptations *in vivo*. Moreover, the initial adaptation of the conduit artery function (FMD%) starts to return to baseline levels as remodelling (DC%)

proceeds. These findings fit the evolving hypothesis, first proposed in animals by Laughlin and colleagues (Laughlin, 1995; Laughlin *et al.* 2003), that functional vascular adaptations precede arterial remodelling in response to exercise training in humans. This hypothesis is reinforced by our findings in the popliteal artery, as endothelial function initially increased, but returned to baseline levels as exercise training continued. In keeping with the upper limb findings, popliteal artery dilator capacity also progressively increased throughout the training period. These novel findings have important consequences for the interpretation of previous exercise training studies and for the future design of intervention trials involving exercise.

Animal data indicate that short-term exercise training enhances vascular function via increases in eNOS and NO bioavailability (Delp *et al.* 1993; Sessa *et al.* 1994; Sun *et al.* 1994; Delp & Laughlin, 1997) while prolonged training induces structural change, sometimes referred to as 'arterial remodelling' (Brown, 2003; Prior *et al.* 2003). It has been hypothesized that such 'structural' remodelling normalizes shear rate levels and may result in NO-mediated endothelial function returning towards initial levels. In our study, brachial artery endothelial function initially increased and 'peaked' after 2 weeks of training. This indicates the endothelial function in the brachial artery rapidly increased, possibly as a direct result of the repetitive increases in shear rate during lower limb exercise training (Green *et al.* 2005). Interestingly, brachial artery endothelial function started to return to baseline levels after just 2 weeks of training, despite the fact that exercise training was continued at the same relatively intensity. Rapid functional adaptation to training and up-regulation of eNOS has previously been reported in animals (Sessa *et al.* 1994; Sun *et al.* 1994; Haram *et al.* 2006). At the end of the 8-week training period, brachial artery endothelial function almost completely returned to baseline.

Brachial artery vasodilator response to an ischaemic exercise stimulus, which was used as a surrogate measure for conduit artery structure (Naylor *et al.* 2005), displayed a gradual increase throughout the 8-week exercise training intervention. This suggests that lower limb exercise training results in a remodelling adaptation, which did not plateau during these 8 weeks. Theoretically, this increase may help to normalize the increased levels of shear rate in the brachial and popliteal arteries. Interestingly, when the time courses of functional and remodelling adaptations in the brachial artery are directly compared, it is evident that remodelling begins after the functional changes reach their peak. This structural adaptation continues across training period, whilst, simultaneously, FMD values return to near baseline levels. These findings provide the first evidence in humans *in vivo* for the hypothesis that conduit artery functional adaptations are superseded by remodelling in response to exercise training.

In keeping with the results found in the brachial artery, changes in popliteal artery function precede adaptations in conduit artery remodelling across the 8-week exercise intervention. Although one might expect to observe vascular adaptation in the directly exercised regions (i.e. popliteal artery) before the relatively inactive beds (i.e. brachial artery) (Thijssen & Hopman, 2008), both arteries in the present study display a similar time course in functional and structural adaptation. During cycling exercise, the change in brachial artery shear pattern in the resting upper limb is linked with endothelial NO release (Green *et al.* 2005). This may contribute to the exercise training-induced increase in brachial artery FMD and also perhaps to longer-term structural adaptations as the artery demonstrates an outward remodelling. However, shear patterns are likely to be different between the brachial and popliteal artery during cycling exercise. Our data suggest that, despite different physiological shear stress stimuli between the limbs (Green *et al.* 2008; Thijssen & Hopman, 2008), both brachial and popliteal conduit artery function

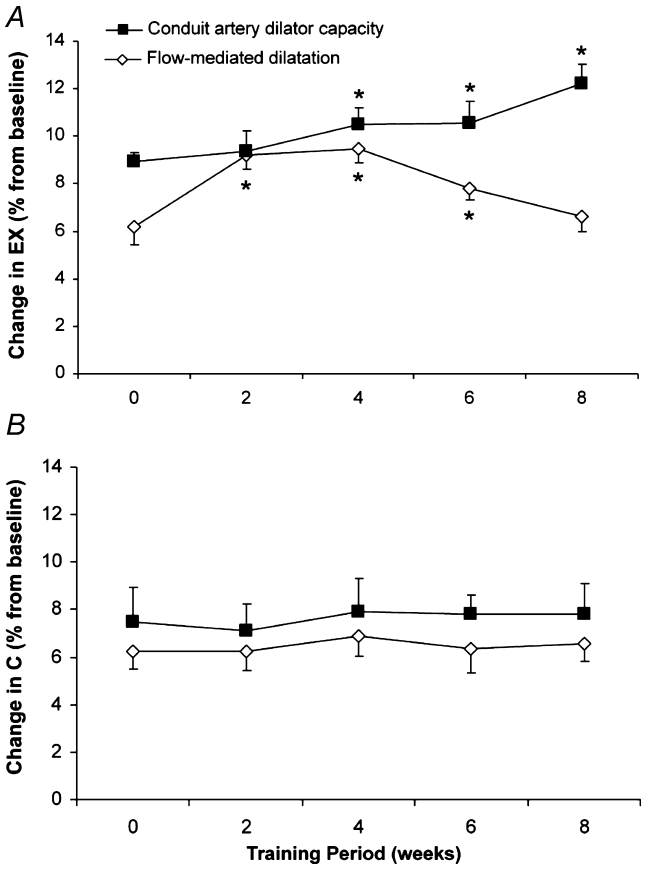

Figure 2. Relative change in popliteal artery diameter from baseline (as a percentage) in response to 5 min ischaemia (flow-mediated dilatation, FMD, ◊) or 5 min ischaemic exercise (vasodilator capacity, DC, ■)
Data are presented before, after and at 2-week intervals throughout an 8-week intervention in exercise trained subjects ($n = 13$, EX) (*A*) and controls ($n = 7$, C) (*B*). Error bars represent S.E.M. $^*P < 0.05$.

and remodelling can adapt with a similar time course in response to 8 weeks predominantly lower limb exercise training.

In contrast to studies that have demonstrated beneficial effects of exercise training on conduit artery function and remodelling in various disease states (Green *et al.* 2004), studies in healthy subjects have reported conflicting results (Kingwell *et al.* 1997*b*; Clarkson *et al.* 1999; Maiorana *et al.* 2001; Ostergard *et al.* 2006; Thijssen *et al.* 2007). Our findings in healthy subjects may help to unravel this conundrum in the literature. Because there is a time course of adaptation in both function and remodelling, choosing to examine vascular function and structure after a fixed intervention period (e.g. 4 weeks or 8 weeks) may result in different interpretations. For example, examining vascular adaptations after 8 weeks' training in our study would result in the conclusion that exercise training in healthy men does not alter conduit artery function, but induces remodelling. This, indeed, is in keeping with the published literature in healthy subjects (Maiorana *et al.* 2001; Ostergard *et al.* 2006; Thijssen *et al.* 2007). In contrast, measuring outcomes at 4 weeks would result in the interpretation that conduit function adapts, without substantive change in remodelling. This too, is consistent with the literature (Hornig *et al.* 1996; Pullin *et al.* 2004). Accordingly, we strongly suggest that more measurements are needed across time when examining vascular adaptations to exercise training in humans. While this statement primarily relates to healthy subjects, it is not unlikely that this also applies to various other groups.

A novel aspect of the current study relates to the method of measuring conduit artery remodelling. Conduit artery vasodilator response to an ischaemic exercise stimulus results in near maximal dilatation (Naylor *et al.* 2005), which was used as an indirect measure for conduit artery vasodilator capacity in the present study. This method of assessment is preferred over measurement of baseline conduit artery diameter used in previous studies, as the latter is more susceptible for fluctuations in sympathetic activity and release of local endothelium-dependent vasodilators and vasoconstrictors. To illustrate this point, Haskell *et al.* (1993) observed no differences in coronary artery cross-sectional area between athletes and their sedentary peers, despite markedly larger responses to pharmacological stimuli which elicited peak dilator capacity in the athletes. Similarly, peak reactive hyperaemic blood flow has been used historically to assess resistance vessel arterial remodelling and most studies indicate that exercise training increases such measures in the absence of change in resting flows. The use of peak dilator stimuli to assess arterial remodelling therefore has a long history in integrative physiology (Folkow *et al.* 1958; Conway, 1963; Folkow, 1978; Takeshita & Mark, 1980; Sinoway *et al.* 1986, 1987; Martin *et al.* 1990; Silber & Sinoway, 1990; Silber *et al.* 1991), although its use to assess conduit arteries is relatively new. Whilst some studies have reported change in resting diameter as an index of structural change with training (Dinenno *et al.* 2001), we suggest that inducing maximal or peak diameter change in conduit arteries provides an index of arterial remodelling which is less susceptible to the influence of functional factors such as the competitive vasoconstrictor or vasodilator tone (Sugawara *et al.* 2007).

There are several limitations to the present study. Exercise programmes which induce greater change in fitness (our changes in $\dot{V}_{O_2,max}$ were modest and we did not observe resting bradycardia), or those which recruit subjects with impaired arterial function *a priori*, would likely be associated with differences in the time course of change in both artery function and remodelling. Our results relating to the time course of change in arterial function and remodelling are also specific to young healthy subjects. Investigations of this time course in different groups and to different exercise stimuli may reveal important new insights into vascular biology.

In conclusion, this study demonstrated the complimentary nature of adaptations in conduit artery function and structure in response to 8 weeks' lower limb training. Our results suggest that functional changes in the conduit arteries precede structural adaptations *in vivo*. The initial increase in upper and lower limb conduit artery function in response to lower limb exercise training is followed by a gradual increase in vascular structure in both vessels. As functional adaptations begin to return to near baseline values, structural adaptations begin to significantly increase across the 8-week intervention period. This arterial remodelling is thought to normalize shear, allowing artery function to return to baseline levels. These findings may explain the disparity in previous literature pertaining to the impact of exercise training in healthy subjects, as previous studies may have missed initial functional changes and not measured the structural adaptations which may have occurred.

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